P2-028 BSTB: Cancer Genetics Posters, Tue, Sept 4

Aberrant methylation of IL-12Rβ2 gene in lung cancer

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Background: Interleukin-12 receptor β (IL-12Rβ2) knock-out mice develop lung adenocarcinoma, and epigenetic silencing by CpG methylation leads to loss of this gene in B-cell malignancies. The aim of this study was to determine whether IL-12Rβ2 methylation is a common feature in human lung cancer.

Methods: We examined mRNA expression of IL-12Rβ2 in lung cancer cell lines, and normal bronchial, and tracheal epithelial cells using RT-PCR, and we examined the methylation status of IL-12Rβ2 in primary lung cancers.

Results: Loss of expression was found in 10 of 13 (77%) NSCLC cell lines, and 2 of 5 (40%) SCLC cell lines compared with normal bronchial or tracheal cells. Treatment of 11 expression-negative cell lines with a demethylating agent restored expression in all cases. Aberrant methylation status of IL-12Rβ2 gene was reversely concordant with its mRNA expression. IL-12Rβ2 methylation was detected in 96 of 230 NSCLCs (42%) and 3 of 6 SCLCs (50%). IL-12Rβ2 rmethylation correlated with poorer prognosis in lung adenocarcinomas (hazard ratio = 2.33, p = 0.0059).

Conclusions: We conclude that epigenetic silencing of IL-12Rβ2 is a frequent event in lung cancers. Aberrant methylation of this gene seems to be a useful predictor of long-term outcome for adenocarcinoma of the lung.

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The role of gene mutations and amplifications involved in epidermal growth factor receptor pathways in non-small cell lung cancer

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Background: Activation of the epidermal growth factor receptor (EGFR) in cancer cells has been shown to promote processes involved in tumor cell proliferation, angiogenesis, invasion, and metastasis, and to inhibit apoptosis. The purpose of the present study is to verify the role of genetic alterations involved in the EGFR pathways in non-small cell lung cancer (NSCLC) development.

Methods: We analyzed mutations of the EGFR, KRAS and PIK3CA genes and amplification of the loci corresponding to those genes in primary tumors from 96 patients with NSCLC. Mutation analyses of EGFR (exon 19 and 21), KRAS (exon 2 and 3), and PIK3A (exon 9 and 20) were performed by the PCR-direct sequencing method. Gene amplification analyses were performed by the fluorescent in situ hybridization method.

Results: EGFR, KRAS, and PIK3CA gene mutations were found in 22 (23%), 2 (2%), and 3 (3%) of 96 NSCLCs, respectively. The copy number gains of these genes were 26 (27%), 10 (10%), and 19 (20%) of them, respectively. On the whole, 55 (57%) of the 96 NSCLCs had one or the other gene alterations. The gene alterations were evenly found in cases with early stage disease as well as in those with advanced disease. It is suggested that these gene alterations play essential roles in the initial step of lung carcinogenesis. EGFR gene mutations were preferentially detected in females (34.1%, 14/41), non-smokers (50.0%, 14/28), and adenocarcinomas (35.1%, 20/57), with statistical significances (Chi-square test, $P < 0.05$), which confirmed previous observations. In contrast, PIK3CA gene amplification were preferentially detected in males (29.1%, 16/55), smokers (34.1%, 16/51), and squamous cell carcinomas (41.3%, 12/29) (Chi-square test, $P < 0.05$). It is suggested that certain carcinogens in the tobacco smoke might have caused PIK3CA gene amplification to promote squamous cell carcinoma development.

Conclusions: The pathway mediated through EGFR, KRAS and PIKCA gene has critical role in the development of NSCLC. NSCLCs can be divided into specific molecular subsets according to the genetic alterations in this pathway.

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NSCLC gene expression study in Estonia

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Cancer is worldwide problem and lung cancer is one of the frequent ones causing ca 1 million deaths every year. Although early stage lung cancer is in most cases surgically curable, about 80% of lung cancer cases due to tumour spread or distant metastases need either radiotherapy, adjuvant or neoadjuvant polychemotherapy. Despite of certain success achieved in the field of combined therapy, the prolonged use of it is limited by developing resistance to drugs and side effects of this treatment.

In everyday lung cancer diagnostics histological classification is used. Accordingly, lung cancer is divided to small cell lung carcinoma (20% of all lung cancers) and non-small cell lung carcinoma (NSCLC, 80% of all lung cancers) including three main groups: squamous cell carcinoma (20-35%), giant cell carcinoma (4.5-15%) and adenocarcinoma (30-50%). Despite of lung cancer histological subgroup diagnostics, the clinical course of the same stage patients is quite different. This fact suggests that histological form of cancer is not sufficient predictor of clinical course of the disease.

The cancer (120) and control samples of the current study originate from Tartu University Hospital and are collected from 28.11.2002 to 31.12.2006. Tissue samples were snap frozen in liquid nitrogen during the operation and processed in RNAlater ICE (Ambion) to eliminate RNA any degradation. RNA was extracted using RiboPure Kit (Ambion) which was followed by RNA quantization (Nanodrop) and quality control on Agilent Bioanalyzer Lab-on-a-Chip technology. RIN value cut-off was set to 7.

In the current study we have used Illumina whole genome gene expression arrays of 47 000 features to monitor molecular patterns of different NSCLC samples in hope to find differentially expressed genes that

would help us to discriminate different NSCLC types and eventually predict the survival and clinical course of the patients.

Our previously performed studies with these tissue samples have shown upregulation of well-known cancer associated genes like STEAP and downregulation of DAPK1, TNFSF10 and EDG1.

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Microarray gene expression in primary lung adenocarcinoma classified by lung asbestos burden

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Background: Asbestos is recognised by the IARC as a human lung carcinogen. The relationship between asbestos and tobacco in lung cancer causation has proven difficult to define, and causal attribution of lung cancer in smokers who have also been exposed to asbestos is often difficult. There are no clinical or pathological characteristics which distinguish lung cancers partly or wholly attributable to asbestos from those due to tobacco alone. The hypothesis for this study was that, despite their phenotypic similarity, lung cancers arising in subjects exposed to respirable asbestos have a different gene expression profile from those arising in subjects with no asbestos exposure.

Subjects and Methods: With institutional ethics committee approval we performed MIAME compliant microarray expression analysis on RNA extracted from resected tumour tissue in 37 cases of adenocarcinoma (AC) on the Operon Human V.2 22k platform. Lung asbestos fibre burden was measured by tissue digestion and filtration, counting ferruginous bodies (FB) by light microscopy. Fibre burdens >20 FB per gram wet weight (/gww) are usually associated with occupational or significant environmental exposure to respirable asbestos (1). Tumour gene expression was compared between 24 subjects with 0 FB /gww (Group 1) and 13 subjects with >=20 FB/gww (Group 2) using Avadis software. Subject groups were similar for age, gender, and smoking. Class prediction was performed in BRB Array Tools v3.5.

Results:

1. Volcano plots identified 21 probes with significantly different expression at p=0.001 and magnitude of absolute fold change >1.5 between these two classes, almost all upregulated in Group 2. The probes corresponded to one pseudogene, ten unknown genes and ten annotated genes including genes in the RAS pathway, novel zinc finger proteins and genes with redox function. The direction of expression difference was validated for 5 out of 6 of these genes by RT-PCR in an independent test set of 30 adenocarcinomas.
2. A 95-gene classifier was developed using the 1 nearest neighbour prediction model by leave one out cross-validation. The mean correct classification rate was 89%, permutation p<0.002. Negative predictive value was 0.852 indicating potential of this classifier to rule out adenocarcinoma related to asbestos exposure. Receiver operating analysis showed that the top 8 genes (by p value) could generate over 90% of the performance of the full classifier.

Conclusions: AC in subjects with lung asbestos bodies showed significant upregulation of several genes compared with AC in individuals without lung asbestos. This finding was confirmed by an independent method in independent subjects, implicating these genes as possible players in asbestos carcinogenicity and / or tumour progression. A 95-gene set was predictive of AC class based upon lung asbestos fibre burden. The performance of an 8-gene subset requires independent evaluation for predictive utility as an RT-PCR panel in clinical and medico-legal settings.

1. Roggli, V. L. and L. L. Sanders (2000). Asbestos content of lung tissue and carcinoma of the lung: a clinicopathologic correlation and mineral fibre analysis of 234 cases. Ann Occup Hyg 44(2): 109-17.

P2-032 **BSTB: Cancer Genetics Posters, Tue, Sept 4**

Human Lung Cancer Related New Genes

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Lung cancer is a leading cause of death worldwide. Lung cancer is becoming the major malignancy in China. Many events including alterations of oncogenes and tumor suppressor genes must have occurred by the time when lung cancer becomes clinically evident. We used the following methods including comparative genomic hybridization; alleotyping; cDNA library construction; and DNA microarray to investigate human lung cancer related genes. On the basis of the above mentioned study, we cloned more than 50 genes, which had never been deeply characterized before.

We identified a novel gene highly expressed in human lung cancer tissue that we named OLC (overexpressed in lung cancer). Forced overexpression of OLC malignantly transformed NIH3T3 cells in vitro and in vivo. Immumohistochemistry staining indicated that OLC was overexpressed in human lung dysplasia/carcinoma in situ and non-small cell lung cancer. The overexpression of OLC was also observed in human esophageal and laryngeal carcinoma. Small interfering RNAs (siRNA) mediated OLC gene silencing in two lung cancer cell lines (H520, H1299) which highly expressed OLC protein, induced significant reduction of cellular growth and high rates of apoptosis. Using an EMSA supershift assay we demonstrated that OLC overexpression can induce translocation of the NF-kappaB complex (p50/p65) from cytoplasm to nucleus in Hela, H520, and H1299 cancer cells. Our preliminary data showed a higher OLC expression in lung squamous cell carcinoma (SCC) of smoker patients than in lung SCC of non-smokers, and cigarette smoke condensate treatment increased OLC expression in human bronchial epithelial cells in vitro. This study indicates that elevation of OLC might be associated with cigarette smoking-related human lung carcinogenesis, particularly at early stage.

Another gene, nominated DENND2D by HGNC, from the novel gene pool constructed in our laboratory was investigated. DENND2D suppressed the malignant transformation of NIH/3T3 cells. Transfection of DENND2D gene into the non-small cell lung cancer cell line, H1299, inhibited the cell proliferation and anchorage-independent growth in vitro, and reduced tumorigenicity of H1299 cell in nude mice. Flow cytometry assay revealed that expression of DENND2D induced G1/S-phase arrest in the cells. Semi-quantitative reverse transcription-PCR demonstrated that down-regulated expression of DENND2D was observed in lung cancer tissue samples, lung cancer cell lines, and the immortalized human bronchial epithelial cell lines, whereas DENND2D remains its expression in the primarily cultured normal bronchial epithelial cells. The lung cancer related novel gene DENND2D may play important role(s) in the pathogenesis of human lung cancers.